New Technique for Closure of Alveolar Cleft With Umbilical Cord Stem Cells

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Abstract: This article presents the case of a newly born female patient with a cleft of the primary palate (alveolar cleft), with an *in utero* diagnosis of the described cleft, from whom umbilical cord stem cells are obtained and cryopreserved. The patient is managed with nasoalveolar molding, and at 5 months of age, she is taken to surgery for cheiloplasty and gingivoperiostioplasty with umbilical cord stem cells. A radiographic and CT follow-up is carried out on the described cleft.

Key Words: Cleft lip and palate, gingivoperiostioplasty, stem cells, umbilical cord stem cells

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There are several ways to classify cleft lip and palate; likewise, there are different treatment options to manage these clefts; however, there is no agreement which determines which is the best treatment option.

In the last 50 years, it has been considered that the facial clefts occur as a result of a deficit of both the mesoderm and the lack of cell migration flowing from the neural tube.[1,2] Patients with complete cleft lip and palate have defects that compromise both the primary and secondary palate, including defects that compromise the alveolar region being of great importance and its management with the restoration of dentoalveolar continuity to provide adequate growth and maxillary stability, support the dental roots on each side of the cleft, and help the eruption of the lateral incisor and canine, in addition to the changes that are produced in the underlying soft and hard tissues.[3,4]

The gingivoperiostioplasty and the use of alveolar bone grafting are the most used procedures for the reconstruction of alveolar clefts in these patients. The primary gingivoperiostioplasty closes the alveolar cleft at the same time that the lip repair is performed, thus avoiding the use of alveolar bone grafting in the future; however, its use is controversial given that the results are inconsistent.[5]

In this preliminary first case report, we discuss the use of umbilical cord stem cells in the primary gingivoperiostioplasty for the management of alveolar clefts and thus avoid the use of alveolar bone grafting in patients with cleft lip and palate in the future.

CLINICAL REPORT

Female patient with an in utero diagnosis through ultrasound of cleft lip and left alveolar cleft. During birth, the collection of the umbilical cord blood stem cell (UCB) is performed.

The UCB sample was obtained after signing the maternal informed consent for banking and the maternal results were negative for VDRL, HBV surface antigen, antibodies against the HBV core antigen, antibodies against Hepatitis C, Human T Cell Lymphotropic Virus (HTVL 1 and 2), and HIV 1 and 2.

At the time of birth, 85 mL of UCB was collected by umbilical vein puncture after the newborn was separated from the cord, but before the placenta was expelled. The UCB processing and cryopreservation was done before 24 hours after its collection.

The process of volume reduction of total nuclear cells was performed through the modified Rubinstein method manually, by centrifugation and separation of the blood components in a closed system of bags to guarantee sterility. The sample was concentrated to 20 mL in the freezing bag, eliminating the plasma portion and a part of the red blood cells.

Before freezing, cell counting was done obtaining 9.9 × 108 of total nuclear cells with 100% viability. The UCB unit was brought to a temperature of 4°C and a cryopreservation solution composed of DMSO/Dextran 40 at a concentration of 10% v/v was added. Subsequently, automated freezing was carried out in a Thermo Scientific chamber where the sample temperature gradually decreased from 4°C to −120°C, 1°C/min. During the procedure, microbiological controls were taken for aerobes, anaerobes, and fungi, which were negative.

The UCB unit was stored in a 25 mL liquid nitrogen-resistant bag with 2 compartments (1 storing 80% and the other 20%). The sample was protected in an overwrap and in a metallic cassette and cryopreserved in vapors of NL2 at −196°C until its use

After birth, treatment begins with nasoalveolar shaping, with the aim of aligning the alveolar segments and leaving the gingiva in contact in both sides to avoid an extensive dissection of the gingivoperiosteal flaps (Fig. 1). At 5 months of age, the patient is taken to surgery, where cheiloplasty is performed along with the gingivoperiostioplasty.

UMBILICAL CORD BLOOD THAWING

The storage compartment of 80% was thawed by heat shock in a water bath at 37°C for 2 minutes until the blood unit was thawed. For the removal of the cryoprotectants, immediately after being thawed, each unit was diluted 1:10 with a solution containing 5% of

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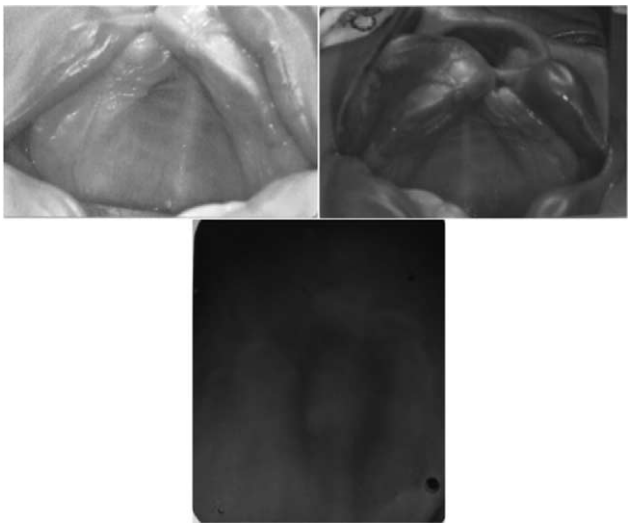

FIGURE 1. Photographs of cleft lip and left alveolar ridge, before and after nasoalveolar shaping with alignment of alveolar segments and contact of the gingiva. Presurgical radiography showing the alveolar cleft.

human serum albumin, 10% HAEMACELL, 1% ACDA in phosphate-buffered saline, and centrifugation was performed for 15 minutes at 1200 rpm.

The cells were resuspended in saline with 5% human serum albumin. The implanted fraction was 7.92 × 108 CNT and 7.19 × 10 5 of CD34 +; the viability was 90%.

Gingivoperiostioplasty was performed intraoperatively with conservative dissection of the gingivoperiosteal flaps, placing 90% of the stem cells in the pocket of the gingivoperiostioplasty using a GELFOAM. Hermetic pocket closure of the gingivoperiostioplasty is performed and cheiloplasty is implemented afterward. At the end of the procedure, the remaining portion (10%) of stem cells is injected into the alveolar and labial surgical wound using a syringe (Fig. 2).

An exclusive liquid diet was formulated for 21 days and then a soft diet for 2 more weeks during the postoperative period. Clinical and paraclinical follow-up of the patient is performed, observing alveolar bone formation in occlusal radiography at 18 months postoperatively, allowing dental eruption, and at 5 years postoperatively by tomography with adequate alveolar height (Fig. 3).

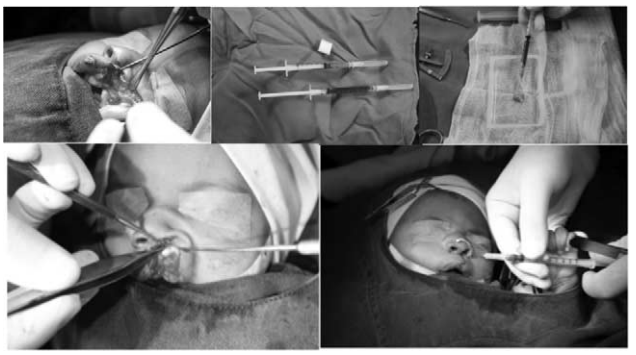

FIGURE 2. Intraoperative photograph of the gingivoperiostioplasty with umbilical cord stem cells and GELFOAM as a device for the placement of stem cells. GELFOAM and cord stem cells in the pocket of the gingivoperiostioplasty. Application of cord stem cells in surgical wound and gingivoperiostioplasty.

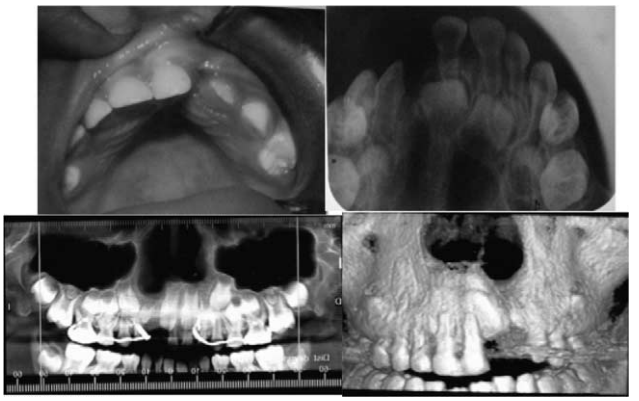

FIGURE 3. Postoperative photograph at 18 months after the surgical procedure. Postoperative occlusal radiography at 18 months with alveolar bone formation. Postoperative tomography at 5 years with bone formation and adequate height in the alveolar cleft.

DISCUSSION

A cleft lip and/or palate rate of 1 in every 1000 live births has been described. The incidence of isolated cleft palate is 1 in 2000 live births and the racial distribution of cleft lip and palate is Asians 2.1:1000, Caucasians 1:1000, and African-derived populations of 0.4:1000.[6] Early identification will give parents a preparation, counseling, and treatment options in the near future for the newborn.[7]

A multidisciplinary team should manage patients with cleft lip and palate. One of the main objectives in the treatment is to optimize their appearance and function. According to the protocol of our service, the surgical repair of the lip takes place between 3 and 5 months of age (the latter in case of nasoalveolar molding) and the repair of the cleft palate is performed between 9 and 12 months of age, due to fact that at this age the phonemic awareness starts developing.

The reconstruction of the alveolar cleft is an essential part in the treatment of patients with clefts because it provides enough support to the nasal base, creates a stable and continuous maxillary arch, performs oronasal fistula closure, eliminates interdental space that allows an adequate eruption of the teeth adjacent to the cleft, and provides an appropriate periodontal support.[2,3]

The use of alveolar bone grafting is the first option among the current treatment options for the management of alveolar clefts given that it is the gold standard with autologous bone graft. To take into account patients who are candidates for alveolar bone grafting, it is important to evaluate the adequate timing for its intervention, the state of the dentition, the age of the patient, and the technique if the patient has unilateral or bilateral cleft lip and palate.

The types of grafts can be classified according to their origin in such as autografts, allografts, or alloplastic material, or according to their composition as medullary or trabecular, cortico-trabecular or cortical; always taking into account bone integration and its principles such as osteogenesis, osteoinduction, and osteoconduction, which are fundamental for proper integration and bone formation after the use of these bone grafts.[2]

Depending on the age in which bone grafting is performed, there can be primary and secondary bone grafting; however, the primary ones are in disuse given to their multiple disadvantages. Secondary alveolar bone grafting is implemented after the closing of the palate and is subdivided into early, between 2 and 5 years (after the eruption of the total temporary dentition); transitional, between 6 and 12 years (during the mixed dentition); and late, in patients >12 years (after the eruption of the permanent canine).

AQ1

The donor sites used for the autografts are the rib, cranial vault, tibia, among others; being the most used iliac crest grafts.[2] However, there may be complications associated with this procedure such as bone resorption, need for multiple interventions, donor-site morbidity (pain, difficulty walking, paresthesias, and injury to important structures), and surgical time, among others. For this reason, other techniques have been described such as alveolar distraction, osteoinduction by recombinant human bone morphogenetic protein, and primary gingivoperiostioplasty.[8]

Skoog in 1965 for the first time describes the use of local mucoperiosteal flaps for the posterior formation of bony bridges within the cleft without morbidity of the associated donor site; however, its use has been controversial in large clefts given the extensive subperiosteal dissection that can be seen associated in alteration of maxillary growth.[8,9] For this reason, Millard in 1990 introduces the use of presurgical orthopedics to reduce the gap of the alveolar cleft in addition to aligning the arch of the maxilla, thus reducing the risk of extensive dissection of the periosteum and the possible associated repercussions.[5,10]

The primary gingivoperiostioplasty removes the tissue between the alveolar cleft and is replaced by gingivoperiosteal flaps for the formation and growth of bone between the cleft, without the need for bone grafting; however, it is not a widely used technique because its results are inconsistent and only approximately 51% have adequate dentoalveolar bone formation in bilateral clefts and 63% have adequate dentoalveolar bone formation in unilateral clefts.[4,9]

Among the objectives expected with this technique are: the closure of the oronasal fistula, the alignment of the maxilla, the stabilization of the premaxilla, improving the symmetry of the nose, allowing the spontaneous eruption of the teeth, and avoiding the use of alveolar bone grafting.[10] The use of nasoalveolar molding should be considered before the surgical procedure to avoid maxillary growth restriction associated with large dissections in large clefts, through which, as its name says, it is possible to mold the cleft, reducing the gap and aligning the axes of the maxilla so that the closure during the gingivoperiostioplasty is optimal and with an adequate aligment between the two maxillas.[2,4,9] Patients who are candidates for this technique should have adequate molding and alignment of the cleft. The primary gingivoperiostioplasty is performed in the same surgical time of the lip repair between 3 and 5 months of age.[3,4,8,9]

Another type of technique for the management of alveolar clefts is the use of recombinant human bone morphogenetic protein for osteoinduction and osteogenesis. Fallucco et al in 2009 published an article regarding their application of this protein to reconstruct alveolar clefts >3 mm. Within the technique, they made a *periosteal pocket* without being the technique mentioned in the gingivoperiostioplasty. These patients were evaluated 6 months after the procedure, showing trabecular bone formation where the protein was applied.[11]

However, in the literature there are no studies or case reports that inform the use of cord stem cells in combination with the primary gingivoperiostioplasty and the results that can be obtained with this technique.

For this reason, it has been decided to publish this preliminary case where it is proposed to use presurgical orthopedics keeping the alveolar segments in contact with the mucosa, which implies that the bone segment is 1 to 2 mm, avoiding extensive dissection of mucoperiostic flaps that may affect the growth of the maxilla; and afterward, cheiloplasty and gingivoperiostioplasty were performed at 5 months of age, which could avoid the use of secondary alveolar bone grafting, achieving the objectives of the mentioned procedure at an earlier age.

Stem cells are a special group of cells that can be classified according to their origin (embryonic and adult) and their differentiation potential (toti-, pluri-, and multipotent).[12] However, all stem cells have 3 common characteristics, namely, self-renewal, indifferentiation, and derivation to any mature cell; these are the lineage (in the case of adult stem cells) that an individual has to regenerate the senescent cells throughout life. Since the age is the same as the age of the newborn, they have a greater potential to regenerate the organism25 with longer telomeres as demonstrated in the hematopoietic progenitors of UCB.[13]

According to their origin, they can be isolated from embryos, which are the so-called embryonic stem cells (ESCs), obtained from the inner cell mass of the embryo in the blastocyststate between days 5 and 6 and develops the 3 embryonic layers: mesoderm, endoderm, and ectoderm.[14] On the other hand, germ cells and adult stem cells (ESCs) are found in all tissues of the body forming the lineage that the body has to replace senescent cells throughout lifetime. Embryonic stem cells are found more frequently in the bone marrow,[15] adipose tissue,[16,17] umbilical cord (UCB),[15,18,19] cord,[14] placenta,[20] olfactory bulb,[21] dental pulp,[14] among others.

Umbilical cord blood is a rich source of hematopoietic progenitor cells, red blood cells, macrophages and granulocytes (CFU-GM), mononuclear cells, CD34 + cells, multipotentials progenitor cells (CFU-GEMM) [22] endothelial progenitor cells,[23] multipotent mesenchymal stromal cells.[24] Since the age is that of the newborn, they have a greater potential to regenerate the organism.[25] With longer telomeres as demonstrated in the hematopoietic progenitors of UCB.[26]

The great heterogeneity of the populations of progenitor cells present in UCB and the different mechanisms of action for cell regeneration have stimulated the development of research that broadens the spectrum of their use in diseases, demonstrating its safety and efficacy; for example, in ischemic diseases such as hypoxic ischemic encephalopathy,[27] stroke,[28] and acute myocardial infarction,[29] and also in osteomuscular diseases in which their regenerative influence has been proved.[30,31,32,33]

The autologous use of UCB as an adjuvant to improve the outcome of reconstructive surgeries generates great expectations not only because the cells have a great potential for differentiation to various mesenchymal tissues, such as bone, cartilage, stroma, and adipose tissue, but also because when used with biomaterials or autologous rich fibrin scaffolds it generates a surgical alternative for reconstructive surgeries.

CONCLUSION

This preliminary case report study indicates that the combination of umbilical cord stem cells and gingivoperiostioplasty during primary cheiloplasty may prevent the performance of secondary alveolar bone grafting.

However, it is necessary to perform this procedure on a large number of patients in a double-blind prospective study to demonstrate that the bone formation described is secondary to cord stem cells and not to 53% to 61% of patients who present bone formation by the gingivoperiostioplasty.

Furthermore, it is necessary to continue monitoring the patients to demonstrate if the alveolar height is adequate, so that secondary alveolar bone grafting is not required.

Therefore, we invite the readers to develop new prospective double blind studies with a reasonable follow-up that could prove our good results.

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